


Auditory Brainstem Circuits That Mediate the Middle Ear Muscle Reflex

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Abstract

The middle ear muscle (MEM) reflex is one of two major descending systems to the auditory periphery. There are two middle ear muscles (MEMs): the stapedius and the tensor tympani. In man, the stapedius contracts in response to intense low frequency acoustic stimuli, exerting forces perpendicular to the stapes superstructure, increasing middle ear impedance and attenuating the intensity of sound energy reaching the inner ear (cochlea). The tensor tympani is believed to contract in response to self-generated noise (chewing, swallowing) and nonauditory stimuli. The MEM reflex pathways begin with sound presented to the ear. Transduction of sound occurs in the cochlea, resulting in an action potential that is transmitted along the auditory nerve to the cochlear nucleus in the brainstem (the first relay station for all ascending sound information originating in the ear). Unknown interneurons in the ventral cochlear nucleus project either directly or indirectly to MEM motoneurons located elsewhere in the brainstem. Motoneurons provide efferent innervation to the MEMs. Although the ascending and descending limbs of these reflex pathways have been well characterized, the identity of the reflex interneurons is not known, as are the source of modulatory inputs to these pathways. The aim of this article is to (a) provide an overview of MEM reflex anatomy and physiology, (b) present new data on MEM reflex anatomy and physiology from our laboratory and others, and (c) describe the clinical implications of our research.

Keywords

acoustic reflex, middle ear muscle, pseudo-rabies virus, auditory prosthetic devices, middle ear ossicles

The middle ear muscle (MEM) reflex is one of two major feedback systems to the auditory periphery. The stapedius and tensor tympani muscles are the target organs of this auditory feedback pathway and are innervated by the efferent fibers originating in the motoneurons around and near the facial or trigeminal nerve nuclei, respectively (Figures 1 and 2; Gelfand, 1998). The MEM reflex increases the impedance of the middle ear by acting on the stapes and the malleus. The MEMs are anatomic antagonists but work synergistically; while the stapedius stiffens the stapes superstructure at the oval window of the cochlea, the tensor tympani exerts forces on the manubrium of the malleus, resulting in an inward deflection of the tympanic membrane (Gelfand, 1984). The medial olivocochlear (MOC) reflex is a parallel pathway to the MEM reflex that provides input to the outer hair cells of the cochlea. Activation of the MOC reflex alters the morphology of outer hair cells, stiffening the basilar membrane and reducing the cochlear amplifier (Liberman & Guinan, 1998).

In some mammals, both the stapedius and tensor tympani muscles contract in response to auditory stimuli. In humans, the stapedius reflex is considered to be the dominant acoustically evoked MEM reflex pathway. Intense, low frequency sound presented to either ear contracts the stapedius in both ears (Figure 1A), similar to the consensual pupillary response to light. Stapedius motoneurons (SMNs) that project to the stapedius muscle likely receive input either directly or

indirectly from the cochlear nucleus, the first relay station for all ascending auditory information originating in the ear. A diversity of inputs descends on SMNs (Figure 1B; D. J. Lee, Benson, & Brown, 2008), and some of these inputs may be nonauditory as a few patients are able to voluntarily contract their stapedius muscle, suggesting cortical projections to SMNs.

Both auditory and nonauditory roles have been attributed to the tensor tympani (Figure 2). The tensor tympani may play a protective role against acoustic trauma and prevent overstimulation from self-generated noise, such as vocalization or swallowing (Klockhoff & Anderson, 1960; Stach, Jerger, & Jenkins, 1984). Though the tensor tympani reflex appears to be activated by intense sounds as part of the startle response and by certain nonauditory stimuli (Djupestrand, 1964, 1967; Klockhoff & Anderson, 1960), its general function in humans is still not completely understood.

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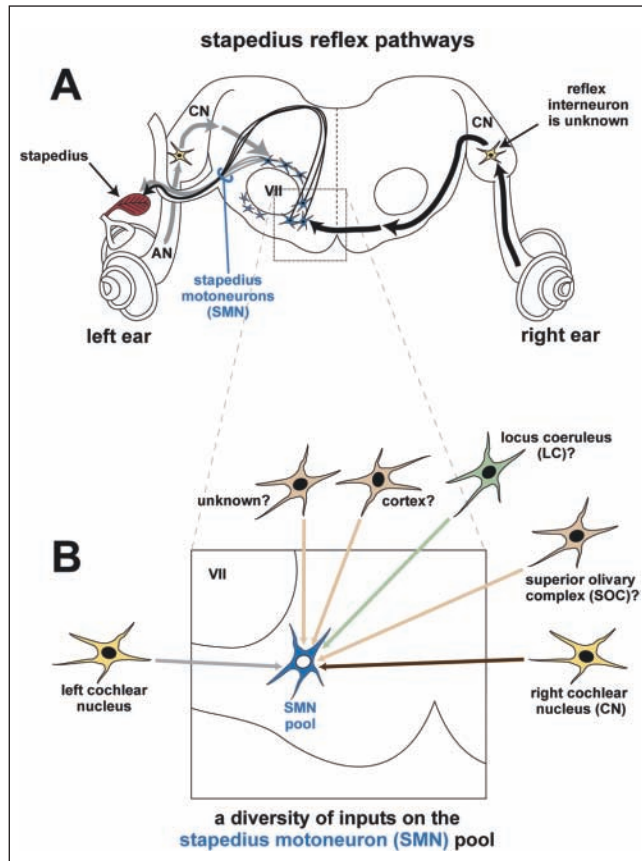


Figure 1. Stapedial reflex pathways

Note: Panel A: The uncrossed stapedius reflex (gray arrows) to the left ear is activated by left ear sound and stimulates the subpopulation of stapedius motoneurons (SMNs) that contract the stapedius muscle of the same ear (left ear). The cochlear nucleus (CN) reflex interneuron of the uncrossed pathway is shown by the large yellow star on the left. It is not clear whether it projects directly to the SMNs or whether there is an intervening synapse. The crossed MEM reflex to the left ear (black arrows) is activated by right ear sound and stimulates the subpopulation of SMNs that contract the stapedius muscle of the left ear. The CN reflex interneuron of the crossed pathway is shown by the large yellow star on the right. The crossed reflex pathway ending with contraction of the right stapedius is not shown. Panel B: Magnified schematic showing the possible diversity of inputs on a representative SMN found ventromedial to the facial nucleus (VII). As shown in Panel A, SMNs respond to sound and likely receive input either directly or indirectly from both CN. Some patients can voluntarily contract their stapedius muscle and so these SMNs must also receive input from cortex. Transneuronal tracing using pseudo-rabies virus (Rouiller, Capt, Dolivo, & De Ribaupierre, 1989; Windsor, Roska, Brown, & Lee, 2007) has shown labeling in the superior olivary complex (SOC), locus coeruleus (LC), and inferior colliculus (IC) after injection into the stapedius muscle in rats. It is unknown whether these regions contain neurons that project directly or indirectly to SMNs.

The spatial organization and light microscopic features of SMNs and tensor tympani motoneurons (TTMNs) have been extensively studied (Guinan, Joseph, & Norris, 1989; Joseph, Guinan, Fullerton, Norris, & Kiang, 1985; D. J. Lee et al., 2008; Mukerji, Brown, & Lee, 2009; Rouiller, Capt, Dolivo, & De Ribaupierre, 1986, 1989; Strutz, Munker, & Zollner, 1988). Differences in the morphological features and location

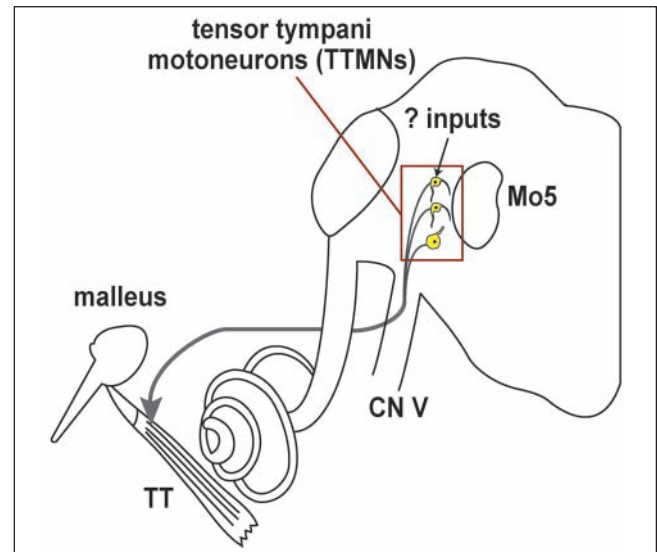


Figure 2. Tensor tympani (TT) reflex pathways

Note: The TT reflex to the left ear is activated by left ear sound and by neurons from elsewhere (?) and stimulates the population of tensor tympani motoneurons (TTMNs) that contract the TT muscle of the same ear (left ear). Based on recent pseudorabies viral tracing studies of the TT reflex by Windsor et al. (2007) and Billig, Yeager, Blikas, and Raz (2007), the interneurons that project from the cochlear nucleus to TTMNs may be multipolar cells located in the anterior ventral cochlear nuclei (AVCN). From the cochlear nuclei, neurons project either directly or indirectly to TTMN pool in rats. Last, efferent fibers (gray arrow) exit the brainstem to innervate the TT via the "nerve to the TT," a branch of the mandibular division of the trigeminal nerve. A range of inputs from the superior olivary complex, serotonergic sources and higher brain centers are thought to descend on TTMNs. The variety of inputs may account for the multifunctional roles of this muscle in response to auditory and nonauditory stimuli (Borg, Counter, & Rosler, 1984; Gelfand, 1984, 1998; Moller, 1984; Nomura, Harada, & Fukaya, 1979; Stach, Jerger, & Jenkins, 1984). The crossed pathway by which the MEM reflex to the left ear is activated by right ear sound is not shown. Mo5, trigeminal motor nucleus; TT, tensor tympani; CN V, trigeminal nerve; TTMN, tensor tympani motoneurons.

of MEM reflex motoneurons may reflect differences in function (Adams, 1986; Kiang, Morest, Godfrey, Guinan, & Kane, 1973; Rhode, Smith, & Oertel, 1983; Rouiller & Ryugo, 1984). Recently, electron microscopic studies in our laboratory have, for the first time, characterized the synaptic profiles of both SMNs and TTMNs (Benson, Brown, & Lee, 2008; D. J. Lee et al., 2008; D. J. Lee, Brown, & Benson, 2009). In this article, we will (a) provide a review of MEM reflex anatomy and physiology, (b) present new data from our laboratory on the central representation of the MEM reflex pathways from our laboratory and others, and (c) describe the clinical implications of our research.

Middle Ear Muscles: A Historical Perspective

The Italian Renaissance (14th-16th century) was a period marked by significant advancements in our knowledge of

temporal bone anatomy, including the discovery of the stapedius and tensor tympani muscles. The first published observations of the MEMs is credited to Italian anatomists Constantius Varolius (1543-1575; Politzer, 1981) and Andreas Vesalius (1514-1564; Vesalius, 1725). Vesalius elevated anatomy to an empirical science (Geiringer, 1970; Mukerji & Lee, 2010) and pioneered anatomic teaching methods as human cadavers gradually replaced animal dissection. Specifically, he made the teaching of anatomy more interactive by using direct observation as the dominant teaching aid. An ardent proponent of Vesalius's systematic approach was the Italian anatomist Hieronymus Fabricius (1533-1619; Cunningham, 1985). Fabricius was one of the first scientists to introduce structure–function relationships in anatomical research. In addition to publishing his observations of venous valves and human fetal formation, Fabricius also proposed the first theories on tensor tympani function. His approach represented a step forward in the study of gross anatomy to understand the “*notia organorum tota*” that is, the entire knowledge of the organ (Smith, Macchi, & Parenti, 2004) and his published observations attributed a protective and a nonauditory role to the tensor tympani muscle (Mukerji & Lee, 2010). These theories have provided the foundation for continued study of MEM function and physiology. The muscular nature of the tensor tympani was later confirmed by the renowned Italian anatomist Bartolomeo Eustachius (1500-1574; Eustachii, 1564).

Anatomy of the Middle Ear Muscles

The stapedius and the tensor tympani are striated muscles located in the middle ear cleft. They are arranged as anatomical antagonists that contract synergistically to decrease sound transmission through the ossicular chain (Figure 3A). The stapedius measures approximately 6 mm in length (Seikel, King, & Drumright, 2000), arises from the pyramidal process of the postero-superior mesotympanum with the tendon attaching to the posterior neck of the stapes capitulum (Moller, 2006; Seikel et al., 2000; Figure 3B). The stapedius consists of a large proportion of Type II muscle fibers that are rich in myosin (Dammeijer, van Dijk, Manni, & van Mameren, 2006), are multinucleated, densely concentrated with ribosomes, and contain a lower concentration of potassium relative to sodium and calcium (Anniko & Wroblewski, 1981). These characteristics enable the stapedius to have a high oxidative capacity that allows it to contract quickly and repeatedly for long periods of time without fatigue (Lyon & Malmgren, 1982). The stapedius is innervated by the stapedial branch of the facial nerve (cranial nerve VII; Politzer, 1861), and contraction results in the posterior rotation and stiffening of the stapes superstructure at the oval window. The tensor tympani is a slender, feather-shaped muscle measuring approximately 25 mm in length (Seikel et al., 2000) and is comprised of short striated muscle cells arranged in parallel fibers (Neergaard, Andersen, Hansen, & Jepsen, 1964),

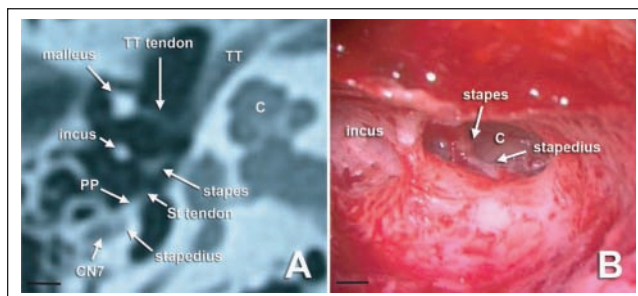


Figure 3. Middle ear muscles in man

Note: Panel A: High-resolution computed tomography (CT) of the right temporal bone, axial plane. In this CT slice, both the stapedius (St) and tensor tympani (TT) are seen in the middle ear, attached to the stapes and malleus, respectively. The St tendon arises from the pyramidal process (PP) and the TT tendon arises from the cochleariform process (not seen). Scale bar: 2 mm. Panel B: Intraoperative photograph, right ear, following completion of a facial recess (posterior tympanotomy) approach to expose the middle ear and cochlea prior to cochlear implantation. The stapedius is clearly seen with the tendon attached to the posterior neck of the stapes superstructure. Scale bar: 5 mm. C, cochlea; CN7, facial nerve.

allowing this muscle to exert tension with minimal displacement (Figure 3A). The tensor tympani have fewer Type II muscle fibers than the stapedius (Lyon & Malmgren, 1988), and more than one third of the tendon contains elastic tissue, dampening the ossicles during excessive stimulation (Lyon & Malmgren, 1988; Neergaard et al., 1964). Abundant amounts of fat are also found in the human tensor tympani, but the physiological significance of this is uncertain (Neergaard et al., 1964).

The tensor tympani originate from the cartilaginous portion of the Eustachian tube, courses through a bony canal in the wall of the anterior middle ear cavity, and attaches to the neck of the manubrium (malleus; Figure 3A; Moller, 2006). A bony partition separates the tensor tympani from the Eustachian tube. The precise reason for this is unclear although it has been theorized that the partition insulates the muscle from vibrations that interfere with sound perception. The tensor tympani is innervated by the “nerve to the tensor tympani” via the otic ganglion (Girardet, 1960), a branch of the mandibular division of the trigeminal nerve (cranial nerve V; Politzer, 1861; Shankland, 2001). There is a dense concentration of motor and proprioceptive nerve fibers in this muscle. These fibers are thinner than other skeletal muscle nerves but still ensure rapid conduction velocities because of their shorter axons (Girardet, 1960). The postsynaptic regions are smaller when compared with stapedial fibers and with less mitochondrial activity in the motor end plates (van den Berge & Wirtz, 1989). Contraction of the tensor tympani muscle pulls the malleus in an anteromedial (inward) direction (Neergaard et al., 1964).

What Is the Role of the MEM Reflex?

The MEM reflex responds to intense acoustic stimuli by increasing middle ear impedance, minimizing acoustic

overstimulation and reducing the masking effects of background noise. Impedance is defined as the ratio of the acoustic pressure over the volume velocity generated by the acoustic pressure (Chien & Lee, 2009; Lilly, 1972). The first electro-acoustic device to measure the acoustic impedance in a clinical setting was developed in the middle of the 20th century and was called the “Metz acoustic bridge” (Metz, 1946, 1952; Thomsen, 1999). Since then, acoustic impedance measurements allow for the indirect monitoring of MEM contraction for both research and diagnostic purposes (Metz, 1946).

A change in acoustic impedance due to contraction of the MEMs results in a decrease in middle ear volume. Reduction in middle ear volume leads to an increase in the middle ear pressure, which is detected as a change in acoustic impedance by a probe inserted into the external auditory canal (Lilly, 1972). The resulting increase in impedance reduces the forward (and reverse) transmission of acoustic energy through the middle ear (Borg, 1971; Borg, Counter, & Rosler, 1984; Borg, Nilsson, & Engstrom, 1983; Moller, 1974). The first observation of an acoustically evoked MEM reflex was made in dogs (Hensen, 1878) and then later in primates (Kato, 1913). Luascher (1930) then reported the first observations of sound-evoked stapedial contractions in humans through perforated eardrums.

Data from acoustic impedance studies have proven that the stapedius is the primary sound-evoked MEM (Borg, 1972; Carmel & Starr, 1963; Liberman & Guinan, 1998; Neergaard et al., 1964). Unlike some animal models, where both the stapedius and tensor tympani contract to sound, the stapedius reflex is the dominant sound-evoked pathway in humans (Zakrisson & Borg, 1974; Liberman & Guinan, 1998; Murata, Ito, Horikawa, & Minami, 1986). Based on this finding, two major functions of the stapedius reflexes have been proposed,

1. Attenuation of acoustic energy reaching the cochlea through the modulation of middle ear impedance (Borg, 1971; Moller, 1974) and
2. Prevention of the masking of speech frequencies through the high-pass filtering of low frequency sound (background noise).

In both instances, the underlying function of the MEM reflex pathway appears to be protective. Contraction of the MEMs results in a frequency-dependent sound attenuation in the presence of intense acoustic stimuli. For example, human ears with an absent stapedius reflex (secondary to a facial nerve palsy) were reported to have suffered from more temporary hearing loss when exposed to noise compared with normal ears with an intact stapedius reflex (Zakrisson, Borg, Liden, & Nilsson, 1980). The MEM reflex also minimizes masking of speech frequencies by intense background noise, which is typically lower in frequency, thereby preserving speech discrimination (Borg & Zakrisson,

1974, 1975; Mahoney, Vernon, & Meikle, 1979; Moller, 1984; Pang & Peake, 1986; Stevens & Davis, 1938). Lastly, the stapedius may also contract to internally generated vocalization to reduce self-stimulation (Borg & Zakrisson, 1975).

In contrast, the tensor tympani is less acoustically responsive in humans than the stapedius. Electromyographic (EMG) recordings of tensor tympani muscles have shown minimal electrical activity in response to sound presentation to both ears (Djupesland, 1964, 1967; Salomon, 1963). Patients suffering from a paralyzed stapedius muscle from facial palsy or stapes surgery (but have an intact tensor tympani function) have absent MEM reflexes (Stach et al., 1984). This finding supports previous observations that the tensor tympani plays a minor role in the MEM reflex pathways to loud sound in humans. Tensor tympani activity through changes in acoustic impedance has been observed during specific nonauditory behaviors. Examples of such behaviors include tactile stimulation of the external auditory canal and face (Klockhoff, 1961), pneumatic pressure on eyelids (Klockhoff & Anderson, 1960), the sudden forced opening of closed eyelids (Terkildsen, 1960), swallowing (Wersall, 1958), and head movements (Carmel & Starr, 1963). Measurable activity of the tensor tympani muscle is also associated with the anticipation of loud sounds (Borg et al., 1984; Gelfand, 1984, 1998; Klockhoff & Anderson, 1960; Terkildsen, 1960) and the startle response (Borg et al., 1984; Gelfand, 1984; Moller, 1984). The tensor tympani is also felt to play an important role in middle ear ventilation based on the close histological and embryological resemblance to Eustachian tube structures, such as the tensor veli palatini (Doyle & Rood, 1980; Kierner, Mayer, & v. Kirschhofer, 2002; Rood & Doyle, 1978; Vacher, Guinan, & Kobler, 1989).

Neuroanatomy of the MEM Reflex Pathways

The MEM reflex begins as sound presented to one ear or both ears (Figures 1 and 2). Following the transduction of acoustic stimuli by the inner hair cells, the action potential is propagated to the first-order neurons (spiral ganglion cells) and the auditory nerve to as yet unidentified interneurons in the ventral cochlear nucleus (VCN; Fekete, 1984; D. J. Lee, de Venecia, Guinan, & Brown, 2006). The cochlear nucleus (CN) is located in the pontomedullary junction of the dorso-lateral brainstem in humans and is the first relay station for all ascending sound information originating in the ear (Adams, 1986; Haines & Lancon, 2003; Harrison & Feldman, 1970; Palmer, 1987). CN interneurons found in the VCN (Billig, Yeager, Blikas, & Raz, 2007; Borg, 1973; D. J. Lee et al., 2006; Windsor, Roska, Brown, & Lee, 2007) project directly (or indirectly) onto MEM reflex motoneurons, located near the motor nuclei of the facial nerve or trigeminal nerve. The neural pathways from the CN interneurons to the motoneurons are still not well-understood. SMNs or TTMNs then

project to the middle ear along branches of the facial or trigeminal nerve, respectively, to innervate the stapedius muscle (stapedial nerve) or tensor tympani (nerve to tensor tympani).

The afferent and efferent components of the MEM reflex have been well described in various animal studies (Borg, 1973; Guinan et al., 1989; Joseph et al., 1985; McCue & Guinan, 1988; Moller, 1984; Strutz, 1981; Strutz et al., 1988; Vacher et al., 1989). The MEM reflex pathways have been shown to consist of three or four neurons (Billig et al., 2007; Borg, 1973; Itoh et al., 1986; Itoh et al., 1987; D. J. Lee et al., 2006; Rouiller et al., 1986; Spangler, Henkel, & Miller, 1982) that comprise an ascending limb (cochlea → auditory nerve → CN interneuron (→ superior olivary complex?) and descending limb (motoneurons → stapedius or tensor tympani; Moller, 1984, 2006; Roeser, Valente, & Hosford-Dunn, 2000; Seikel et al., 2000). To date, the identification and organization of CN interneurons participating in the MEM reflex pathways have not been fully characterized. Viral transneuronal techniques have indicated both a direct connection between the CN and the MEM reflex (Billig et al., 2007; Itoh et al., 1987) as well as the presence of an additional synapse after the CN (Rouiller et al., 1986, 1989; Windsor et al., 2007). Similar to the direct projections observed in the MOC auditory efferent pathways (Thompson & Thompson, 1991; Ye, Machado, & Kim, 2000), the possibility of a direct link between the CN interneurons and MEM reflex is controversial (Itoh et al., 1986; Rouiller et al., 1986). Another aim of current research is to characterize the MEM reflex motoneurons in greater detail on the basis of their location, morphological features, and dendritic characteristics.

MEM Reflex Motoneurons

The efferent pathways that comprise the MEM reflexes are well-described. SMNs innervate the stapedius muscle via the stapedial branch of the facial nerve (seventh cranial nerve), whereas the TTMNs innervate the tensor tympani through the “nerve to the tensor tympani,” a branch of the mandibular division of the trigeminal nerve (fifth cranial nerve). The light microscopic features of MEM motoneurons have been characterized in cat (Friauf & Baker, 1985; Guinan et al., 1989; Joseph et al., 1985; McCue & Guinan, 1988; Mizuno et al., 1982; Spangler et al., 1982; Vacher et al., 1989), rat (Rouiller et al., 1986, 1989), guinea pig (Mizuno et al., 1982; Strutz et al., 1988), and mouse (Mukerji et al., 2009). The location and spatial organization of MEM motoneurons differ between SMNs and TTMNs.

Stapedius Motoneurons

SMNs are located in close proximity to the facial motor nuclei bilaterally (Figure 4; Joseph et al., 1985; Rasmussen, 1946; Rouiller et al., 1989; Strominger et al., 1981; Windsor et al.,

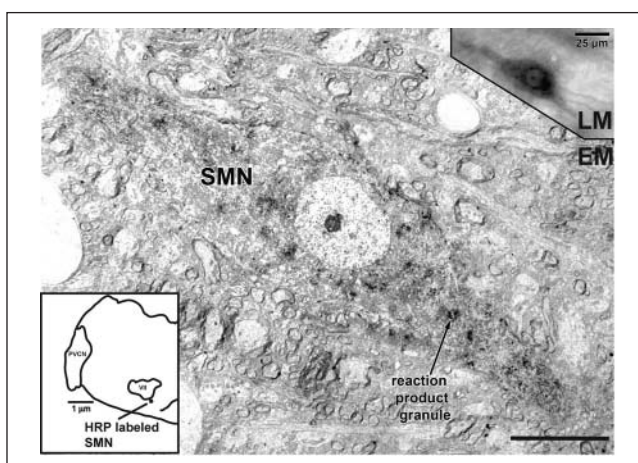


Figure 4. Horseradish peroxidase (HRP) labeled stapedius motoneuron (SMN) in a rat model

Note: Center image: low magnification electron micrograph of HRP-labeled SMN with black reaction product granules (scale bar = 10 μm). Lower left inset: drawing of the left half of a coronal section of rat brainstem demonstrating the location of the labeled SMN ventro-medial to the facial nerve motor nucleus (VII). PVCN-posteroventral cochlear nucleus. Upper right inset image: bright-field photomicrograph of the same epoxy-embedded labeled SMN.

Source: D. J. Lee et al. (2008). Reprinted with permission from the Association for Research in Otolaryngology.

2007) and are distributed across the perifacial and periolivary regions in the cat (Lyon, 1978), and ventromedially and dorsomedially to the facial nuclei in guinea pigs (Strutz et al., 1988). SMNs have been reported to respond exclusively to either ipsilateral, contralateral, or bilateral acoustic stimulation (McCue & Guinan, 1988; Vacher et al., 1989) and are spatially organized around the facial motor nuclei according to their physiological responses to sound (Joseph et al., 1985; Lyon, 1978; Shaw & Baker, 1983). A similar spatial arrangement of neural innervation to the muscle fibers of the stapedius has not yet been described (Wiener-Vacher, Guinan, Kobler, & Norris, 1999).

In cat, the stapedius has a higher innervation ratio than the tensor tympani (Blevins, 1964; Joseph et al., 1985). For instance, close to 1,100 SMNs in cat are found in the perifacial and peri-olivary regions of the brainstem (Joseph et al., 1985), and these SMNs supply approximately 1,730 stapedius muscle fibers. The stapedius therefore has an innervation ratio of 1:1.6 (Blevins, 1964; Wiener-Vacher et al., 1999).

In guinea pig and rat, SMNs are located both ventromedially and dorsomedially, relative to the motor facial nucleus (Rouiller et al., 1989). They are spatially organized according to their physiologic responses to sound presented to the ipsilateral or contralateral ear (McCue & Guinan, 1988; Vacher et al., 1989). SMNs are felt to receive nonauditory inputs as well. The MEMs contract in response to self-generated vocalization (Borg & Zakrisson, 1975) to minimize self-stimulation. Serotonergic terminals on SMNs may modulate activity of

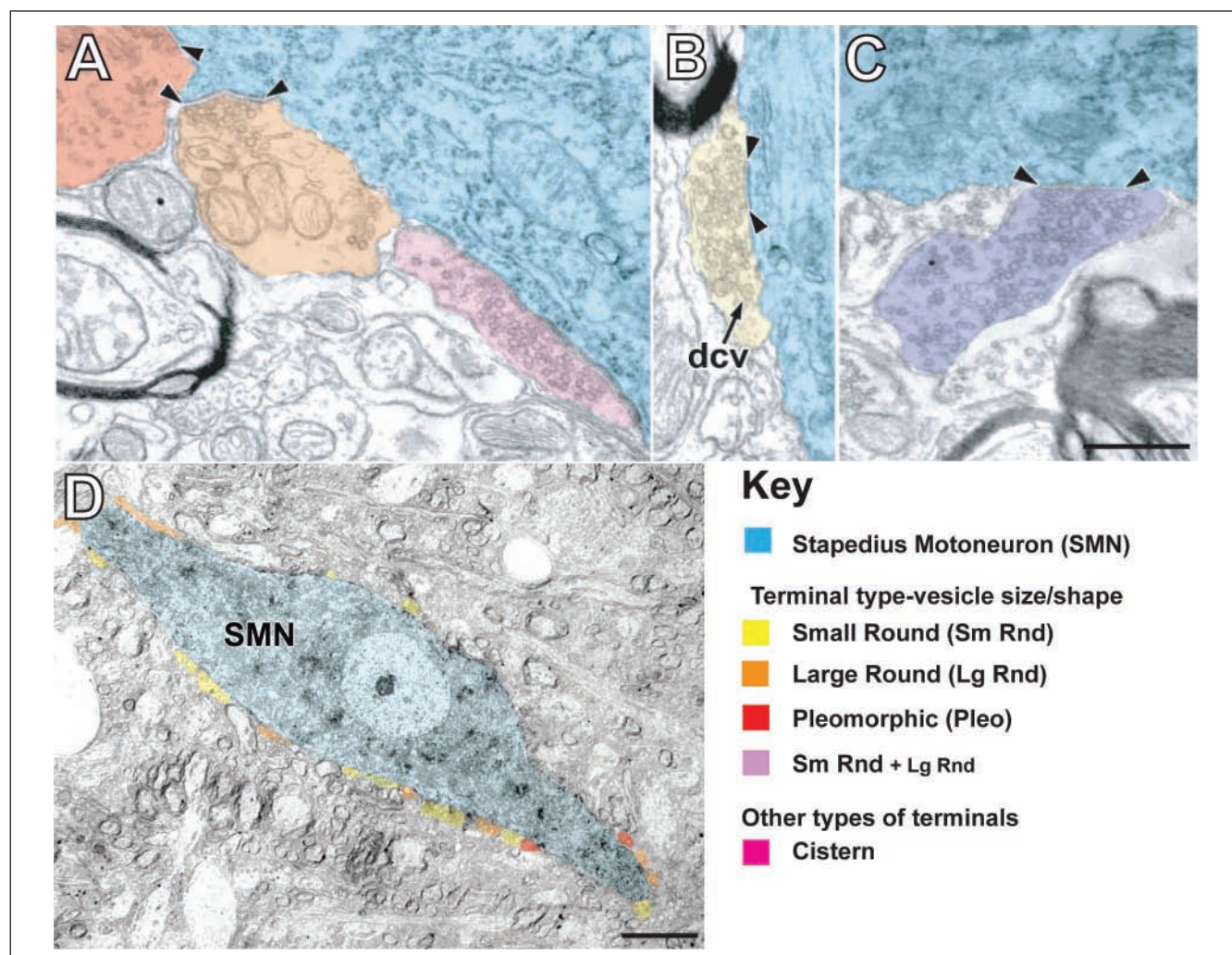


Figure 5. Electron micrographs of the major terminal types seen on the stapedial motoneuron (SMN) from Figure 4. Note: Five types of synaptic terminals (see key) found on SMNs (cyan). Panel A: Synaptic terminals with pleomorphic vesicles (Pleo, red) and large, round vesicles (Lg Rnd, orange). Panel B: Synaptic terminal with small, round vesicles (Sm Rnd, yellow) and dense core vesicles (DCV). Panel C: Synaptic terminal with round vesicles of different sizes (Het Rnd, lavender). Panel D: Low magnification electron micrograph demonstrating the distribution of synaptic terminals on the SMN cell body and proximal dendrites. Synaptic terminals are color coded according to vesicle shape and size. The proportion of membrane in contact with synaptic terminals was approximately 50%. Scale bar = 0.5 μ m (A-C), scale bar = 10 μ m (D). Source: D. J. Lee et al. (2008). Reprinted with permission from the Association for Research in Otolaryngology.

the stapedius reflex (Thompson, Thompson, & Britton, 1998). Finally, higher brain centers may provide cortical control over contraction of the stapedius muscle (Borg et al., 1984; Gelfand, 1984; Gelfand, 1998; Moller, 1984; Nomura, Harada, & Fukaya, 1979; Stach et al., 1984). The sources of these descending inputs to SMNs are not known.

We hypothesized that the diversity of inputs on SMNs would be reflected in a variety of terminal types on these motoneurons. The ultrastructural features of synaptic terminals on retrogradely labeled SMNs in rat were studied using electron microscopy (D. J. Lee et al., 2008). In SMNs, both the proximal dendrites and SMN cell bodies were seen to be densely and evenly populated with synaptic terminals (D. J. Lee et al., 2008). A variety of inputs on SMNs with both excitatory and

inhibitory properties were described. These terminals were classified into five major types according to the size and shape of their synaptic vesicles (Figure 5; D. J. Lee et al., 2008). The most common synaptic terminal type contained small, round vesicles (Figures 5B and 5D; D. J. Lee et al., 2008), and these were felt to be excitatory (Uchizona, 1965). Similar terminals containing small round vesicles were observed on MOC neurons (Benson & Brown, 2006), and the source of these terminals were multipolar neurons residing in the CN. It is uncertain whether SMNs receive inputs from multipolar neurons or other neurons found in the CN (Benson & Brown, 2006).

The second most common synaptic terminal type on SMNs contained large, round vesicles (Figure 5A; D. J. Lee

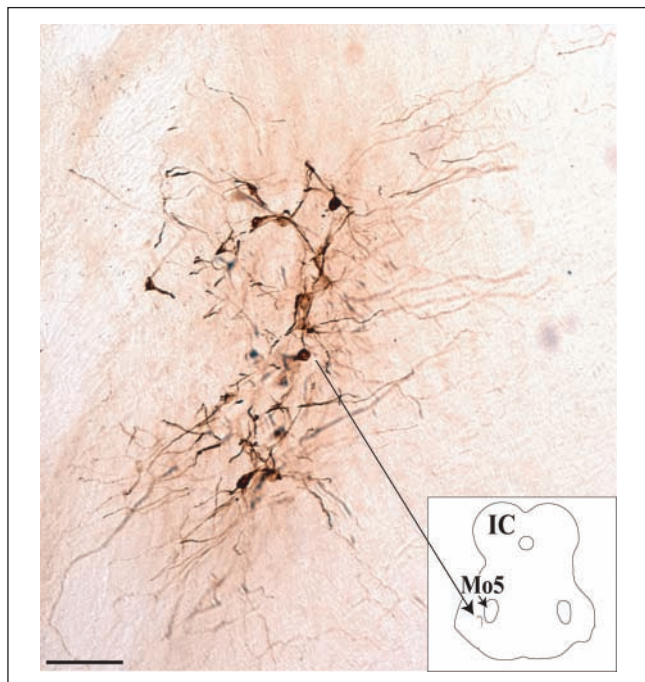


Figure 6. Fluoro-gold (FG)-labeled tensor tympani motoneurons (TTMNs) in a mouse model

Note: FG-labeled TTMNs were arranged as a curved column ventrolateral to the trigeminal motor nucleus (Mo5; see inset). IC, inferior colliculus. Scale bar: 100 μ m.

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et al., 2008). These large round terminals ranged in size and one putative source of these inputs on SMNs is the globular bushy cell in the CN. Smith, Joris, Carney, and Yin (1991) described projections of globular bushy cells to the caudal brainstem near the facial motor nucleus where SMNs are located (Smith et al., 1991). In their study, large round vesicles were also seen in terminals of globular bushy cells. Our study revealed additional terminal types on SMNs (Figure 5A). The complex integration of inputs by SMNs reflected by the diversity of synaptic terminals seen in our ultrastructural study of SMN terminals may account for the multifunctionality of the MEM reflex (D. J. Lee et al., 2008).

Tensor Tympani Motoneurons

In cat, it has been demonstrated that only 700 TTMNs supply approximately 4,000 tensor tympani muscle fibers (Blevins, 1964; Shaw & Baker, 1983) to give the tensor tympani an innervation ratio of 1:5.7. TTMNs are found immediately ventrolateral to the trigeminal motor nuclei (Billig et al., 2007; Itoh et al., 1986; Lyon, 1978; Mizuno et al., 1982; Mukerji et al., 2009; Rouiller et al., 1986; Spangler et al., 1982; Strominger et al., 1981; Windsor et al., 2007; Figure 6). The location of TTMNs are thought to correlate to “Cell Group K,”

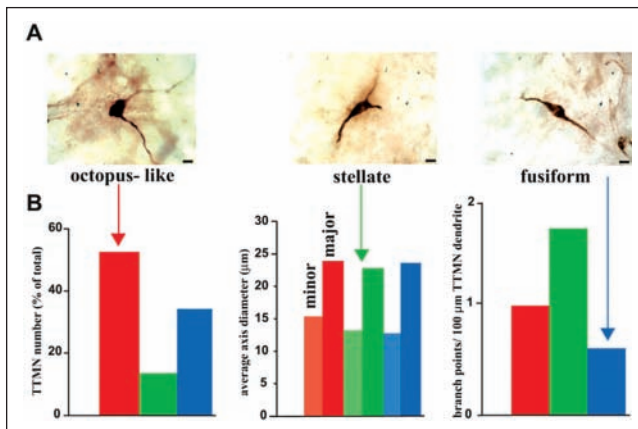


Figure 7. Tensor tympani motoneuron (TTMN) subtypes

Row A: Micrographs of three fluoro-gold labeled TTMN subtypes found in mice; “octopus-like” (red), “fusiform” (blue) and “stellate” (green). Scale bars: 10 μ m. Row B: Left graph shows the total percentage of each TTMN subtype; Most subtypes were octopus-like (52%) and least were stellate (7%). Fusiform were 41%. Middle graph shows the average minor axis diameter (light color) and major axis diameter (dark color) of each TTMN subtype; octopus-like TTMNs were the largest whereas stellate TTMNs were the smallest. Right graph shows the average number of branch points in the first 100 μ m of primary dendritic length for each TTMN subtype; stellate TTMNs branched the most.

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a specific area in the brainstem containing motoneurons that supply the masseter, digastric, and Eustachian tube muscles (Donga, Dubuc, Kolta, & Lund, 1992; Reuss, Kuhn, Windoffer, & Riemann, 2009; Saad, Dubuc, Westberg & Lund, 1999). Within this region, TTMNs are arranged as a crescent-shaped column (Mukerji et al., 2009; Rouiller et al., 1986) extending rostrally to the medial aspect of the lateral lemniscus (Billig et al., 2007; Spangler et al., 1982). This pattern of distribution is described as being distinct from the trigeminal motor nucleus in terms of location, cell body size, and dendritic spread (Friauf & Baker, 1985). For this reason, an area exclusively consisting of TTMNs is considered to be a separate “tensor tympani motor nucleus of V” rather than an accessory of the trigeminal motor nucleus (Friauf & Baker, 1985; Hutson, Glendenning, & Masterton, 1979). Unlike SMNs, TTMNs have not been shown to be spatially located around the trigeminal motor nuclei according to their individual physiological responses to stimuli.

Recently, our group studied the morphology and dendritic characteristics of mouse TTMNs using a retrograde neuronal tracer called Fluoro-Gold (Mukerji et al., 2009). Each TTMN was categorized into one of three subtypes, based on the number and orientation of the primary dendrites that projected from its cell body (Figure 7). “Octopus-like” TTMNs (most commonly found) had two dendrites projecting from one side of the cell body. “Stellate” TTMNs (least commonly found) had three or more dendrites projecting from the cell body and “fusiform” TTMNs had two dendrites projecting

from opposing poles of the cell body (Mukerji et al., 2009). The different TTMN subtypes may reflect the variety of auditory and nonauditory inputs that trigger TT contraction and are similar to the range of neuron cell body morphology in the CN (Adams, 1986; Kiang et al., 1973; Rhode et al., 1983; Rouiller & Ryugo, 1984).

The orientation and distribution of dendrites contribute to the ability of a neuron to receive and respond to stimuli (Bergquist & Ludwig, 2008; Berkowitz, Riabowol, & Gilman, 1989; Hickmott & Ethell, 2006; Sotnikov, 2005; Saxon & Hopkins, 2006). In our mouse study, TTMNs formed sparsely branched dendrites that radiated in all directions (Mukerji et al., 2009; Figure 8). TTMN dendrites directed dorsomedially were more frequent and were the longest, some extending for distances longer than 600 μm (Figure 9). A similar orientation and length bias exists for medially projecting dendrites of MOC neurons that receive inputs from the contralateral side of the brainstem in mice (Brown & Levine, 2008).

Since TTMNs dendrites appear to project well beyond the immediate region of labeled motoneurons (Friauf & Baker, 1985; Mukerji et al., 2009), they present a large surface area on which to receive synaptic inputs. For example, Friauf and Baker (1985) observed a mean of five heavily branched primary dendrites emanating from each labeled TTMN in cat. These dendrites traveled for long distances and radiated into areas near the superior olivary complex (SOC; Friauf & Baker, 1985). In both cat and mouse, TTMN dendrites avoid crossing the trigeminal motor nucleus (Friauf & Baker, 1985; Mukerji et al., 2009). The separate and distinct organization of TTMN dendrites further supports the presence of physiological differences between TTMNs and the motoneurons of the trigeminal motor nucleus (Friauf & Baker, 1985).

The proximal dendrites of TTMNs are known to receive the majority of synaptic input compared with the cell body, which is relatively sparsely innervated (D. J. Lee et al., 2008). These TTMN terminals receive inputs from the CN (Billig et al., 2007), serotonergic sources (Thompson et al., 1998), and higher cortical areas (Gelfand, 1984, 1998), all of which mediate the MEM reflex during auditory and nonauditory behaviors.

The synaptic terminals on retrogradely labeled rat TTMNs were recently investigated using electron microscopy in our laboratory (D. J. Lee et al., 2008; Figure 10). The frequency and morphology of terminal types observed on TTMNs were similar to that seen on SMNs (Benson et al., 2008; D. J. Lee et al., 2008). Synaptic terminals on TTMNs were classified into the four terminal types according to the size and shape of the synaptic vesicles. They were named (a) large round, (b) small round, (c) pleomorphic, and (d) heterogeneous. Compared with SMNs, there were slightly fewer small round terminal types found on TTMNs (D. J. Lee et al., 2008). SMNs also received a rare Cistern-type terminal that was not seen on TTMNs (D. J. Lee et al., 2008). In TTMNs, terminal types with round vesicles (large round and small round) were

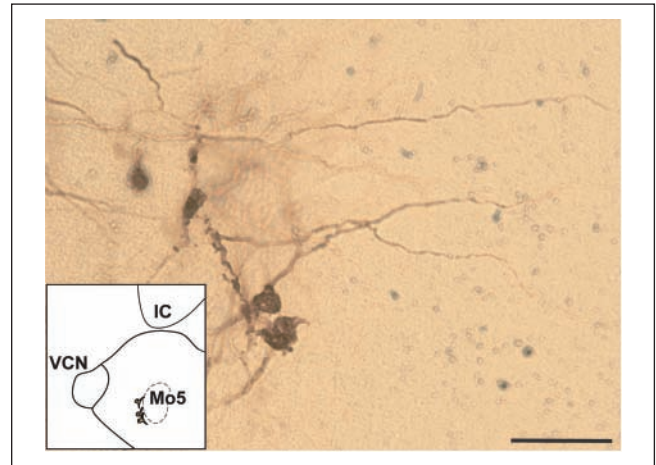


Figure 8. Fluoro-gold (FG)-labeled tensor tympani motoneuron (TTMN)

Note: Dendrites extended medially toward the trigeminal motor nucleus (Mo5; toward right; see inset). Micrograph is a montage of two images taken of a single section at different focal planes. Scale bar: 50 μm .

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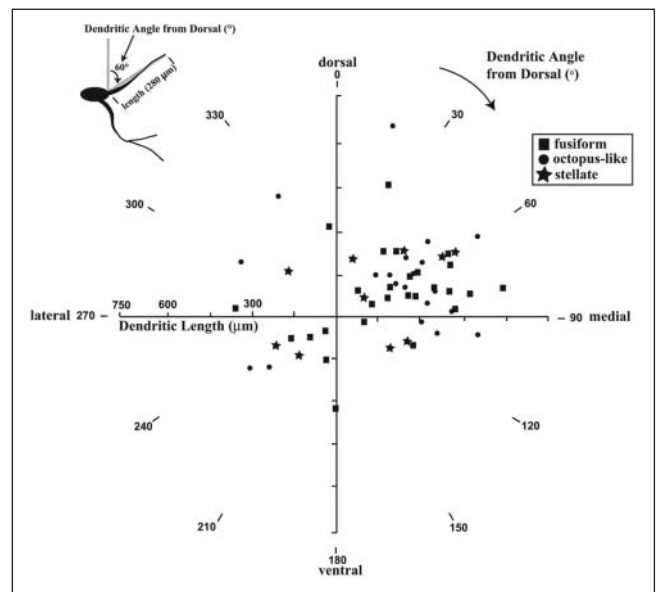


Figure 9. Polar plot of tensor tympani motoneuron (TTMN) dendritic length and orientation

Note: Polar plot shows the lengths (μm) and dendritic angles ($^{\circ}$) of reconstructed fluoro-gold labeled dendrites across each TTMN subtype; octopus-like (circles), stellate (stars), and fusiform (squares). Dendrites directed dorsomedially were more numerous and were the longest in length. Inset shows measurement of dendritic angle by drawing a line from soma to dendrite tip and measuring the angle of this line with respect to the dorsal direction.

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most common (Figure 10) accounting for approximately 80% of the terminal types (D. J. Lee et al., 2008). This is a much higher percentage than that found on spinal motoneurons

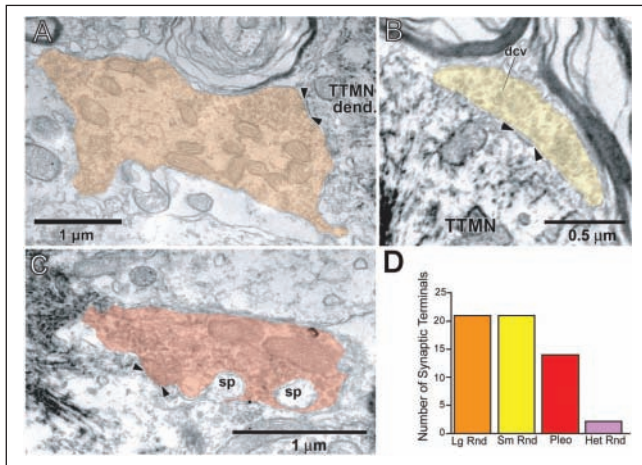


Figure 10. Electron micrographs of major round vesicle terminal types seen on tensor tympani motoneurons (TTMNs; D. J. Lee et al., 2008) in a rat model

Note: Synapses are indicated with arrowheads. Panel A: Terminal with large round (Lg Rnd) vesicles. Myelin of axon is also indicated. Panel B: Terminal with small round (Sm Rnd) vesicles. A dense core vesicle (DCV) is seen occasionally. The round vesicles of Lg Rnd terminals are noticeably larger than the round vesicles of Sm Rnd terminals. Both of these terminal types have asymmetric synapses and are thought to be excitatory. Panel C: Terminal with pleomorphic (Pleo) vesicles. A third of terminals with pleomorphic vesicles engulf spines (SP) from the TTMN. Vesicles of pleomorphic terminals have various shapes from flat to round. These terminals have symmetric synapses and are thought to be inhibitory. Panel D: Graph showing frequency of appearances of different synaptic terminals on TTMNs.

(40% to 45%; Conradi, Kellerth, Berthold, & Hammarberg, 1979). The relatively high proportion of terminal types containing round vesicles signifies that the TTMNs receive mainly excitatory input (Uchizona, 1965). The presence of some pleomorphic terminal types (Figure 10C) suggests that TTMNs also receive some inhibitory input. As in the case of SMNs, the assignment of TTMN terminal types to the cell bodies of origin has not been established. The CN, SOC, serotonergic sources, and higher cortex are all possible sources (Billig et al., 2007; Gelfand, 1984; Stach et al., 1984; Thompson et al., 1998).

Quantitative Analysis of MEM Motoneuron Synaptic Terminals

Until recently, the classification of synaptic terminal types was based on the subjective assessment of vesicle shape and size, which could be affected by factors such as packing density and comparison to vesicles in surrounding terminals (D. J. Lee et al., 2009). The quantification of morphometric differences between synaptic terminal types would assist in a more precise assignment of each terminal type to a possible input source. Recent ultrastructural studies of synaptic terminals on MEM reflex motoneurons further studied the differences between terminal types based on synaptic vesicle morphometry (D. J. Lee et al., 2009).

Morphometric studies of MEM reflex motoneuron terminals that include the mean vesicle area (nm^2) support the earlier subjective classification of the three common terminal types; small round, large round, and pleomorphic (D. J. Lee et al., 2009). Based on similar quantitative measurements, SMNs and TTMNs were shown to receive terminals of similar morphology. These motoneurons receive three common types of terminals as well as two rare types of terminals; those with heterogeneously sized vesicles and another terminal type packed with large dense core vesicles (DCVs; Benson et al., 2008; D. J. Lee et al., 2008, 2009). Because SMNs and TTMNs are found in separate regions of the brainstem and are unlikely to receive identical inputs, these observations suggest that similar neurotransmitters are used in these parallel MEM reflex circuits.

Differences were seen between SMN and TTMN terminal types. For example, most large round terminals of SMNs did not contain DCVs but in TTMNs, some large round terminals did contain DCVs (Benson et al., 2008; D. J. Lee et al., 2008). Perhaps, these differences reflect underlying functional differences, because the DCVs found in TTMN terminals (~80 nm diameter) may be associated with synapse assembly and plasticity (Sorra, Mishra, Kirov, & Harris, 2006).

The surface areas of large round vesicles in SMN terminals (D. J. Lee et al., 2009) corresponded to a diameter comparable with similar vesicles found in globular bushy cell terminals in the medial nucleus of the trapezoid body (MNTB; superior olivary complex; Jean-Baptiste & Morest, 1975). Projection studies showed that labeled globular bushy cell axons projected to the caudal parts of the SOC and perifacial areas, a region containing SMNs (Spirou, Brownell, & Zidanic, 1990). There, they terminate in large endings (Smith & Brezinova, 1991) similar to the biggest large round terminals observed on SMNs (Benson et al., 2008; D. J. Lee et al., 2008).

Both SMN and TTMN neurochemistry has also been studied in great detail (Reuss, Al-Butmeh, & Riemann, 2008; Reuss et al., 2009). SMNs use calcitonin gene-related peptide (CGRP) as a cotransmitter to acetylcholine (Ach; Reuss et al., 2008). In contrast, TTMNs do not express CGRP but produce nitric oxide instead (Reuss et al., 2009). TTMNs also use acetylcholine, bombesin, cholecystokinin, and endorphin in addition to nitric oxide. Additionally, both SMNs and TTMNs were found to be closely related to structures chemically associated with neuroactive substances, such as substance P and serotonin (Reuss et al., 2008, 2009; Thompson et al., 1998). Serotonin is believed to modulate MEM contractions during certain nonauditory activities such as chewing (Ramirez, Ballesteros, & Sandoval, 2007; Thompson et al., 1998) and during the varied arousal states of animals such as cats (Friauf & Baker, 1985) and monkeys (Kita, Chiken, Tachibana, & Nambu, 2007). Immunohistochemical studies suggest that TTMNs have serotonergic positive nerve endings (Thompson et al., 1998). Rouiller et al. (1986) reported on multisynaptic serotonergic connections between raphe

nuclei in the brainstem and TTMNs (Rouiller et al., 1986). Raphe nuclei are a cluster of cells located in the brainstem that contribute to the reticular formation, a higher brainstem center controlling behavior and arousal states through serotonergic activity (Seikel et al., 2000; Siegel, Roeling, Gregg, & Kruk, 1999). The close relationship of the serotonergic system and the MEM reflex supports the theory that tensor tympani contraction is triggered by nonauditory inputs. Although the efferent pathways from motoneurons to MEMs are well-characterized, fundamental unknowns continue to exist, which demand further query and research. Specifically, the multiple central circuits (reflex interneurons) that mediate the auditory and nonauditory responses of the MEMs are not fully understood.

The Identity and Location of the MEM Reflex Interneurons

The identity and organization of MEM reflex interneurons in the CN has been the focus of only a few studies (Billig et al., 2007; Borg, 1973; D. J. Lee et al., 2006; Lyon, 1978; Rouiller et al., 1986, 1989). The major anatomical subdivisions of the CN include the dorsal cochlear nucleus (DCN), anteroventral cochlear nucleus (AVCN), and posteroventral cochlear nucleus (PVCN; Fitzgerald, Gruener, & Mtui, 2007; Palmer, 1987). Specific subtypes found in each subdivision of the CN display a variety of somatic and dendritic characteristics (Brawer, Morest, & Kane, 1974; Osen, 1969). For instance, the ventral cochlear nucleus (VCN) is known to contain spherical bushy cells, globular bushy cells, multi-polar cells, and octopus cells (Adams, 1983, 1986, 1989; Osen, 1965, 1969, 1970). The differences in morphology among cell types in the CN is associated with unique physiologic responses to auditory stimulation (Adams, 1986; Cohen, Brawer, & Morest, 1972; Evans & Nelson, 1973; Osen, 1969, 1970; Pfeiffer, 1966; Rhode et al., 1983) and projections to different targets in the auditory brainstem (Harrison & Warr, 1962; Schofield & Cant, 1996a; Van Noort, 1969).

Early anatomical studies have suggested that CN interneurons reside within the VCN (Borg, 1973). This was demonstrated by measuring the effects of surgical brainstem lesions on the acoustic impedance in rabbits (Borg, 1973). However, these lesions were nonspecific and may have damaged auditory nerve fibers of passage, confounding the results. In addition, these studies did not attempt to identify the CN interneurons (Borg, 1973). Recently, our group has used both anatomic and physiologic techniques in a rat model to localize CN interneurons. The integrity of the MEM reflex pathway was assessed using a reflex metric (D. J. Lee et al., 2006). A reflex metric is a method of quantifying the strength of a physiologic response by subjecting an experimental model to a stimulus and then measuring the reflex response. In this study, the reflex assay was the suppression of sound evoked "distortion product otoacoustic emissions" (DPOAE) in one ear in response to a reflex eliciting sound stimulus in

the contralateral ear (Azeredo, Woods, Sterns, & Relkin, 2000; Relkin, Sterns, Schipper, Azeredo, & Woods, 2001; Smith, Sterns, Prieve, & Woods, 2005). The DPOAE is a measurement of sound ($2f_1 - f_2$) following the presentation of two fundamental frequency tones f_1 and f_2 (Kemp & Chum, 1980). The DPOAE requires normal forward and reverse acoustic transmission through the middle ear. Contraction of the MEMs reduces the stimulus and the emission associated with the DPOAE. The DPOAE was eliminated following the surgical transection of the MEMs, indicating that the assay was specific for the MEM reflex (Relkin et al., 2001).

We used this reflex assay to localize the MEM reflex interneurons in the CN. In the first set of animals, we surgically transected the "acoustic striae" of the auditory brainstem ipsilateral to the sound stimulus (D. J. Lee et al., 2006). Acoustic striae are pathways in the auditory brainstem through which CN interneurons are thought to exit the CN. The three striae are named in relation to their location as they exit the CN and are called "ventral," "intermediate," and "dorsal" (Borg, 1973; Held, 1893; Masterton & Granger, 1988; Moller, 1984). Sectioning of the dorsal and intermediate acoustic striae (the ventral acoustic striae was preserved) ipsilateral to the sound stimulus did not result in the elimination or the reduction of DPOAEs in the opposite ear (D. J. Lee et al., 2006). These findings suggest that the CN interneurons are likely to exit the CN through the ventral striae. Because ventral striae are closely related to the VCN, the results support Borg's initial finding that the location of CN interneurons is likely in the VCN (Borg, 1973; D. J. Lee et al., 2006). The predominance in the ventral striae of projections from globular bushy cells makes this cell type a possible candidate for a MEM reflex CN interneuron (Friauf & Ostwald, 1988; D. J. Lee et al., 2006; Smith et al., 1991).

In the second set of animals, we focally lesioned different regions of the CN using a neurotoxic compound called Kainic acid. Kainic acid was selected because it destroys cell bodies but preserves the nerve fibers of passage (Coyle, Molliver, & Kuhar, 1978; McGeer & McGeer, 1978; Wuerthele, Lovell, Jones, & Moore, 1978). The DPOAE was measured in response to contralateral sound before and after lesioning of the DCN, PVCN, or AVCN. Postexperiment histology was examined and correlated with alterations in the MEM reflex strength (Bird, Gulley, Wenthold, & Fex, 1978; de Venecia, Liberman, Guinan, & Brown, 2005; Melcher & Kiang, 1996). Lesions of all subdivisions were associated with transient reductions in the DPOAE and therefore MEM reflex strength. A long-term reduction of the MEM reflex was seen in one case in which the injection was made in the VCN, and this correlated with neuronal loss in the PVCN, suggesting that the CN interneurons reside here (D. J. Lee et al., 2006). Additional data from our laboratory (data not published) with additional animals have shown that lesions of the VCN are associated with a reduction in MEM reflex strength. Interestingly, the VCN is also a region common to the MOC reflex pathways and acoustic startle reflex

interneurons. The MEM, MOC, and startle reflexes are all triggered by sound and provide additional antimasking effects to the auditory periphery (Liberman & Guinan, 1998).

Transneuronal Labeling of MEM Reflex Pathways Using Pseudo-Rabies Virus

Modified neurotropic viruses with decreased virulence have become powerful tracers for transneuronal labeling. Bartha pseudo-rabies virus (PRV) is an attenuated alpha herpesvirus that was originally developed for the pig industry as a vaccine against pseudorabies infection, but has a variety of properties that make it an ideal choice for studying the central auditory system as a transneuronal viral tracer. PRV has diminished cytopathogenicity, a broad host range, and demonstrates extensive staining of neuronal cell bodies and their dendrites (Card, 2001). Unlike conventional tracers, PRV can label chains of synaptically linked neurons by retrograde transport of viral particles across synapses that link these neurons. Many studies have shown that PRV can invade axons and replicate, and then migrate in a retrograde fashion to transneuronally infect multisynaptic circuits in the brain (Boldogkoi, Bratincsak, & Fodor, 2002; Card, 2001; Helfferich, Uherezky, Boldogkoi, Vitez, & Palkovits, 2003; Loewy, 1998; Sams, Jansen, Mettenleiter, & Loewy, 1995).

Direct Projections of CN Interneuron to Motoneurons

Work by Billig et al. (2007) has used PRV to determine the location and identity of the tensor tympani reflex interneurons in the CN. Following injection of the tensor tympani with PRV, bilateral transynaptically labeled neurons (mean neuronal counts: eight ipsilateral, five contralateral) in the dorsal and dorsomedial regions of the AVCN were seen (Billig et al., 2007; Figures 11 and 12A). Labeled neurons were also found in the dorsal PVCN, although to a lesser extent (Figure 12B). Based on both morphological appearance (large somas and orientation of dendrites that radiate across the CN; Doucet & Ryugo, 1997) and location in the AVCN, the majority of labeled neurons were classified as "radiate multipolar cells" (Billig et al., 2007). Radiate multipolar neurons have been previously shown to project to the dorsal cochlear nucleus (DCN) on the same side (Doucet & Ryugo, 1997; Schofield & Cant, 1996b) and are similar in appearance to the large commissural neurons that traveled to the contralateral CN (Cant, 1982; Doucet, Ross, Gillespie, & Ryugo, 1999; Schofield & Cant, 1996a; Wenthold, 1987). Billig et al. (2007) observed that the number of labeled neurons increased bilaterally in the AVCN at longer survival times (69 to 71 hours; means of 21 ipsilateral vs. 30 contralateral neurons). A few neurons were also detected in the PVCN and DCN, centered mostly along the border between the two regions (Billig et al., 2007). These findings represent the first description of putative MEM reflex interneurons in the CN.

Preliminary results from a similar series of time-graded survival experiments by Windsor et al. (2007) demonstrated labeled CN interneurons 48 to 53, 62 to 72, and 96 to 120 hours following injection of PRV into rat stapedius and tensor tympani muscles. For stapedius injections, the majority of labeled neurons were seen bilaterally in the rostral half of the PVCN but also within regions clustered around the PVCN and DCN, with a mean neuronal count of four ipsilateral and five contralateral (Figure 13A). Minimal labeling was seen bilaterally in the AVCN and auditory nerve root (Windsor et al., 2007). Tensor tympani injections, however, exhibited a different labeling pattern in which CN neurons were seen bilaterally in the rostral half of the dorsal AVCN (Figure 13B; Windsor et al., 2007) similar to the work of Billig et al. (2007). No labeling was seen in the PVCN, DCN, or auditory nerve root following tensor tympani injections.

The difference in locations of CN interneurons between the stapedius and tensor tympani alludes to the possible existence of two separate groups of reflex interneurons in the CN involved in the MEM reflexes, namely, those in the PVCN controlling the stapedial reflex and those in the AVCN controlling the tensor tympani reflex. Observations by Billig et al. (2007) also strongly support the presence of direct fibers from the VCN bilaterally to the TTMNs based on the temporal course of viral replication (Billig et al., 2007). Consistent labeling was noted in the VCN (without SOC labeling) at survival times ranging between 48 and 62 hours, suggesting that the tensor tympani reflex may also consist of three neurons: cochlea → VCN → motoneurons (Billig et al., 2007). Direct connections between the CN and the motoneurons, without involvement of an additional synapse have been demonstrated, though the physiological relevance of a direct connection is still unclear (Billig et al., 2007; Borg, 1973; Itoh et al., 1987; Ito & Honjo, 1988; Van Noort, 1969). For instance, Itoh et al. (1987) provided evidence of direct afferent fibers projecting from the VCN to the TTMNs in cats after injecting a retrograde tracer into the pontine tegmentum, a region in the brainstem containing TTMNs. Labeled neurons were observed bilaterally in the DCN and VCN (Itoh et al., 1987). Similar direct projections have been observed for other descending auditory pathways, such as the MOC reflex (Thompson & Thompson, 1991; Ye et al., 2000). It is not clear whether there is a similar direct projection of CN interneurons to stapedial motoneurons.

Based on the tonotopical organization of the VCN (Saint Marie, Morest, & Brandon, 1989) and the cochleotopical organization of the auditory nerve fibers in the VCN (Fekete, Rouiller, Liberman, & Ryugo, 1984; Noda & Pirsig, 1974), the spatial arrangement of radiate multipolar CN interneurons controlling the tensor tympani could occupy the high frequency regions of the VCN (Fekete et al., 1984; Liberman, 1991). The minimal labeling of neurons found in the auditory nerve supports the involvement of the tensor tympani in the

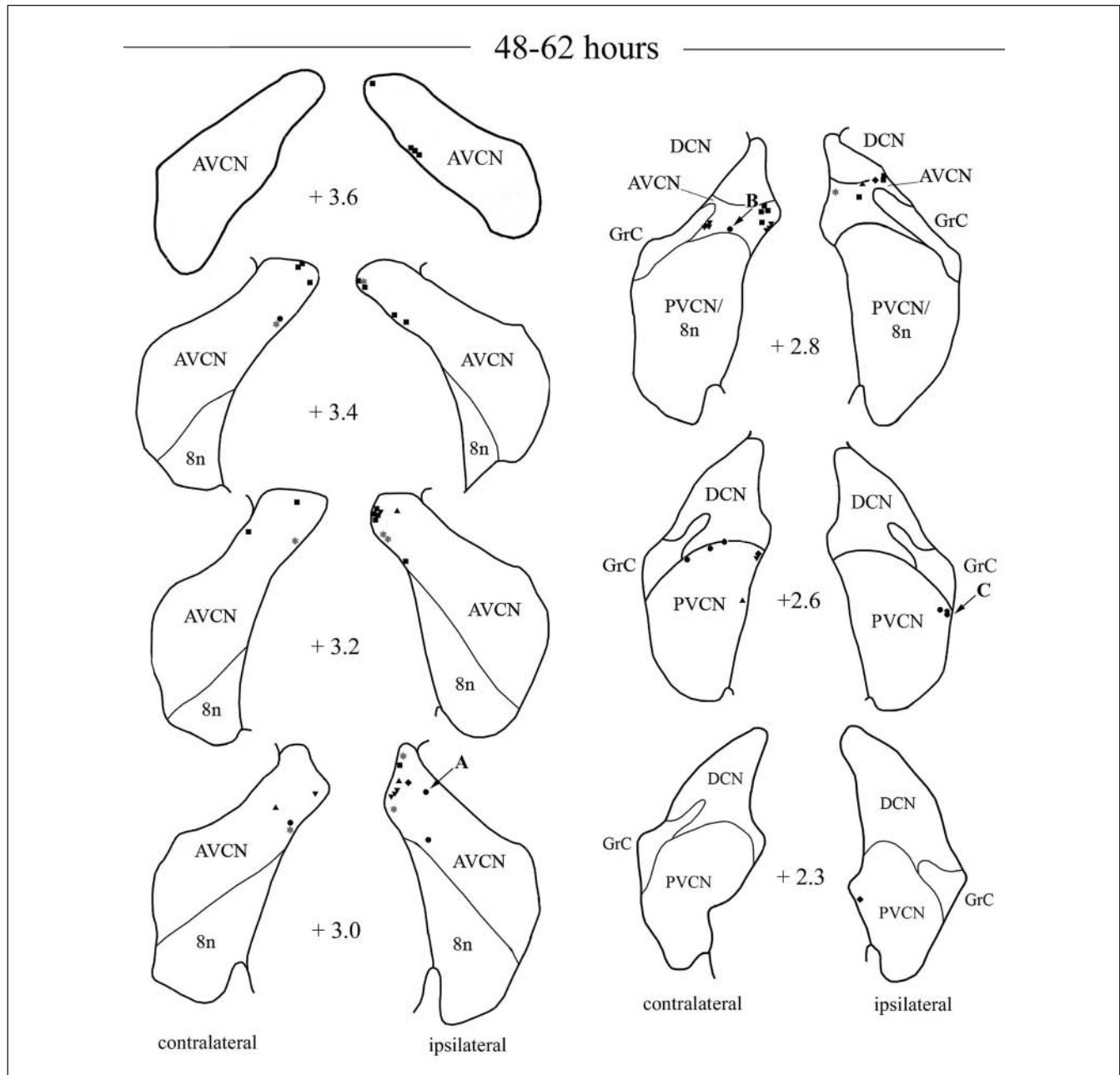


Figure 11. Camera Lucida drawings of ventral cochlear nucleus (VCN) labeling

Note: Brainstem atlas shows the locations of pseudo-rabies virus (PRV)-labeled neurons in the ventral cochlear nucleus (VCN) following injection into the right tensor tympani muscle. Survival times ranged from 48 to 62 hours. Each symbol represents a labeled neuron. Neurons on right side of each diagram are located ipsilateral to the PRV injection. The number between diagrams indicates the relative rostrocaudal distance in mm of the section from the obex. Arrows with A-C letters indicate the locations of infected neurons shown in Figure 13. 8n, vestibulocochlear nerve; AVCN, anterolateral cochlear nucleus; DCN, dorsal cochlear nucleus; GrC, granular layer of cochlear nuclei; PVCN, posteroventral cochlear nucleus.

Source: Billig et al. (2007). Reprinted with permission from *Brain Research*.

acoustic startle reflex (Y. Lee, Lopez, Meloni, & Davis, 1996). Projections of CN interneurons directly onto the MEM reflex motoneurons have been described as being similar to those seen in the MOC pathway (Thompson & Thompson, 1991; Ye et al., 2000).

Indirect Projections of CN Interneuron to Motoneurons

Previous studies using transneuronal tracers (Rouiller et al., 1986, 1989) have revealed an additional synapse in the SOC

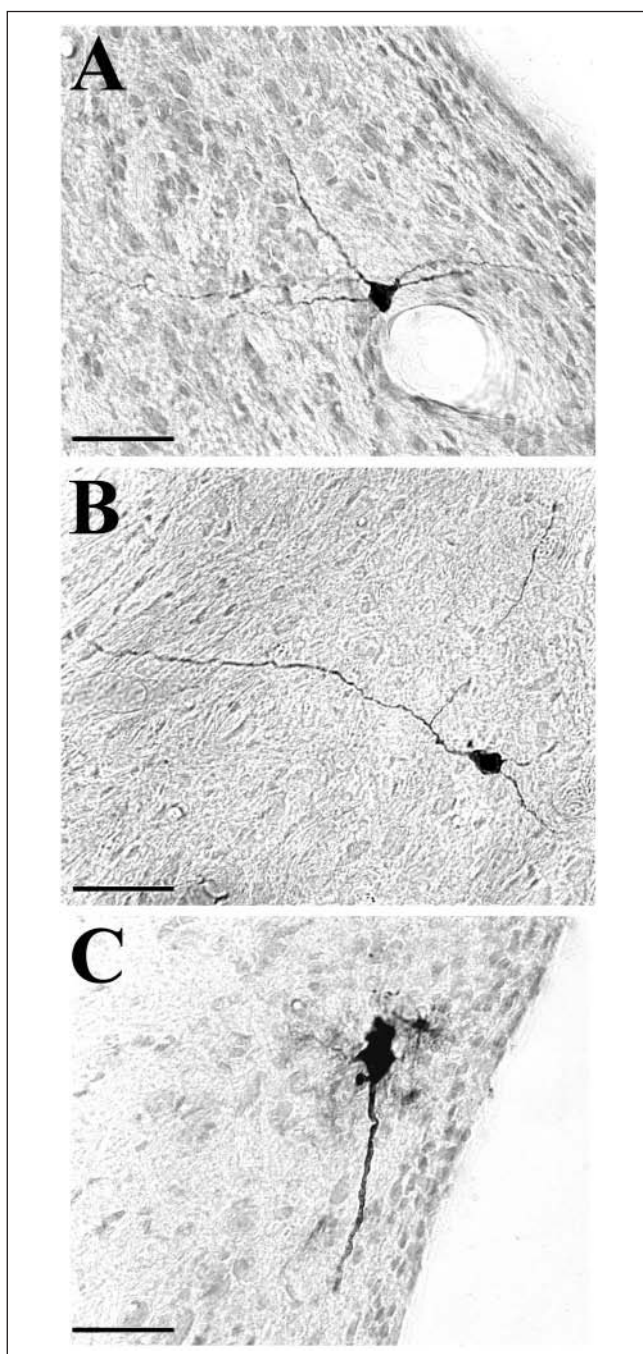


Figure 12. Pseudo-rabies virus (PRV)-labeled ventral cochlear nucleus (VCN) neurons following injection into the tensor tympani muscle

Note: Panels A and B: Radiate multipolar cells in the anteroventral cochlear nucleus (AVCN). Panel C: Large cell at junction of the posteroventral cochlear nucleus (PVCN) and the Schwann-cell border of the cochlear nerve. Scale bars represent 50 μ m.

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that might be involved in the central pathways of the MEM reflex. Specifically, after injecting herpes virus suis into the rat stapedius muscle, Rouiller et al. (1989) described labeled

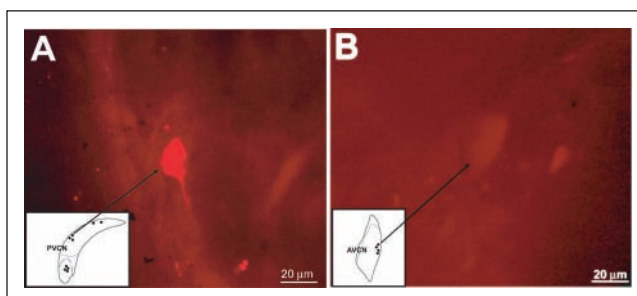


Figure 13. Middle ear muscle reflex interneurons labeled with pseudo-rabies virus (PRV) found in the posteroventral and anteroventral cochlear nucleus (PVCN and AVCN, respectively; Windsor et al., 2007) after 62 hours

Panel A: Fluorescence microscopy showing a left-sided PVCN neuron (see inset) following injection of the left stapedius muscle with PRV. Panel B: Fluorescence microscopy showing left-sided AVCN neurons (see inset) following injection of the left tensor tympani muscle with PRV.

neurons at the level of the SOC in the mediotrapezoid region. In a similar experiment, transneuronally infected neurons appeared close to the SOC bilaterally after injection of herpes virus suis into rat tensor tympani (Rouiller et al., 1986). These findings suggest that some CN interneurons traveled indirectly to MEM motoneurons by way of the SOC. However, labeled neurons in these early viral studies did not represent the entire network of neurons involved in the MEM reflex pathways as CN interneurons were not labeled in these experiments.

Billig et al. (2007) detected a small number of labeled cells bilaterally in the MNTB of the SOC as early as 62 hours following the injection of PRV into the rat tensor tympani (Table 1). There was a clear ipsilateral dominance, with some labeling also seen in the periolivary (PO) cell groups. More obvious labeling in both the MNTB and the dorsal, medioventral, and lateral aspects of the PO cell groups was observed following inoculation times between 69 and 71 hours. After 69 hours, Billig et al. (2007) reported a mean of 38 ipsilateral labeled neurons compared with 18 contralateral labeled neurons in the SOC. After a survival time of 78 to 80 hours, the number of labeled neurons in the SOC increased to a mean of 462 ipsilateral versus 246 contralateral neurons (Billig et al., 2007). On the basis of their morphology, the labeled neurons were characterized as “marginal cells,” “microneurons” and other cell types that could not be classified (Billig et al., 2007). The presence of these cell types in the SOC was deemed significant because prior studies have shown that marginal cells in the VCN project to the PO cell groups and the contralateral MNTB of the SOC (Friauf & Ostwald, 1988). Additionally, neurons originating in the PO and MNTB have also been shown to project to the TTMNs on both sides in rabbit (Borg, 1973) and rat (Rouiller et al., 1986).

Preliminary results from PRV injections into rat stapedius and tensor tympani muscle (Windsor et al., 2007) also

Table 1. Cases of Injections of Pseudorabies Virus Into Single Tensor Tympani (TT) Muscles

Cases	Survival Times (Hours)	Labeled Motoneurons	Labeling in Cochlear Nuclei	Labeling in the Superior Olivary Complex
TT 1	48		—	—
TT 2	48			—
TT 3	48			—
TT 4	62			—
TT 5	62			—
TT 6	62			—
TT 7	62			
TT 8	62			
TT 9	69			
TT 10	70			
TT 11	70			
TT 12	71			
TT 13	78			
TT 14	80			

Note: Cases are represented with their respective survival times. Shaded areas indicate brain regions in the acoustic reflex pathways that contained labeling.

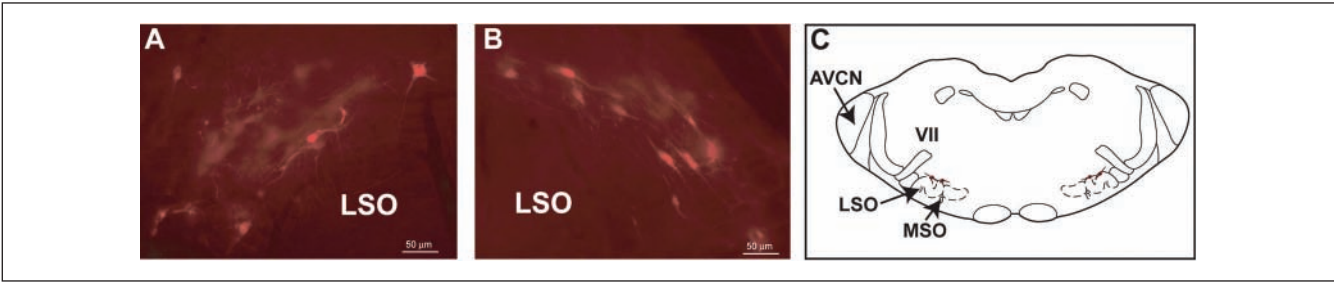


Figure 14. Bilateral superior olivary complex (SOC) labeling following pseudorabies virus (PRV) injection into the left tensor tympani (Windsor et al., 2007)
Note: Fluorescence microscopy shows bilateral periolivary neurons (A: left, B: centre). Most of the PRV-labeled SOC neurons had two major dendrites arising from an elongated cell body, and were located immediately caudal to the region of labeled TTMNs (C: right). LSO = lateral superior olive; MSO = medial superior olive; AVCN = antero-ventral cochlear nucleus; VII = seventh cranial nerve.

showed transneuronally labeled neurons in the bilateral PO cell groups dorsal and medial to the lateral superior olive (LSO) 48, 62, and 96 hours following an injection but not to the extent and increasing pattern as described by Billig et al. (2007; Figure 14).

Involvement of the SOC suggests that one possible MEM reflex circuit diagram would include four neurons: cochlea (spiral ganglion cell) → VCN (reflex interneuron) → SOC (superior olivary complex) → motoneuron. The SOC is a relay station for ascending and descending auditory information traveling to and from both ears. Some of the fiber tracts leaving the CN send collaterals to the SOC before forming the lateral lemniscus, a specialized tract of axons that carries acoustic information from the CN to various brainstem nuclei and the midbrain (Kelly, van Adel, & Ito, 2009; Saldana, Aparicio, Fuentes-Santamaria, & Berrebi, 2009). It is located medial to the CN in the caudal portion of the pons and consists of three main nuclei, namely the medial superior olive (MSO), the LSO, and the MNTB. The MSO receives inputs from bushy cells of both CN and is sensitive to interaural

time differences. The LSO receives excitatory inputs from the bushy cells of the ipsilateral CN, inhibitory inputs from the contralateral CN, and responds exclusively to interaural amplitude differences (Fisher & Harrison, 1962; Harrison & Warr, 1962; Oliver & Beckius, 1996; Ollo & Schwartz, 1979). Through the information processed by both nuclei, the SOC collectively assists in sound localization by analyzing time and amplitude differences between acoustic signals. There are also between six and nine PO cell groups interspersed around the three main nuclei.

The SOC plays an as yet undefined role in the MEM reflex pathway. Early mechanical degenerative studies have demonstrated that CN interneurons synapsed onto third-order neurons in the SOC on the same and opposite side of the stimulated ear (Borg, 1972, 1973). This was based on surgical lesions made in the medial part of the SOC that affected both the contralateral and the ipsilateral sound evoked MEM reflex in rabbit (Borg, 1972, 1973; Moller, 1984).

There are possibly coexisting indirect pathways (Moller, 1984; Shlomo & Silverman, 1991; Wiley & Block, 1984;

Wilson, Shanks, & Lilly, 1984) that are multisynaptic and travel in parallel to the main MEM reflex arc. These pathways are largely unidentified and are likely to involve the extra-pyramidal system (Courville, 1966). For example, anatomical degenerative studies have demonstrated that when lesions of the reticular formation are made rostral to the SOC, there is degeneration in the facial motor nuclei (Borg, 1973). Compared with the direct pathways, the indirect pathways are slower and more sensitive to medications such as barbiturates and dependent on the arousal state of the organism (Baust, Berlucchi, & Moruzzi, 1964; Borg & Moller, 1975; Salomon, 1963). The presence of several pathways involved in the MEM reflex may explain the multi-functionality of the tensor tympani and stapedius muscles in response to a wide array of auditory and nonauditory behaviors.

Clinical Implications

Acoustic reflex testing has been used to facilitate the diagnosis of disorders of the middle ear (Jerger, Harford, & Clemis, 1974), cochlea (Olsen, Noffsinger, & Kurdziel, 1975), vestibulocochlear nerve (Anderson, Barr, & Wedenberg, 1969a), and brainstem (Jerger & Jerger, 1975). Specifically, measurements of the stapedial reflex can help to discriminate between otosclerosis and ossicular discontinuity (Anderson & Barr, 1971; Anderson, Jepsen, & Ratjen, 1962; Ebert, Zanation, & Buchman, 2008; Maurizi, Ottaviani, Paludetti, & Lungarott, 1985) and distinguish between cochlear and retrocochlear pathologies (Anderson, Barr, & Wedenberg, 1969b; Callan, Lasky, & Fowler, 1999; Chiveralls, Fitzsimmons, Beck, & Kernohan, 1976; Hunter, Ries, Schlauch, Levine, & Ward, 1999). The stapedial reflex can identify patients at risk of eighth cranial nerve tumors (Anderson et al., 1969b; Jerger & Hayes, 1983; Olsen et al., 1975), determine whether a facial nerve lesion is infra- or suprastapedial (Djupestrand, 1976; Fee, Dirks, & Morgan, 1975) or identify a pathology of the central auditory system, such as an acoustic neuroma (Jerger, 1980; Jerger & Hayes, 1983; Jerger & Jerger, 1975; Jerger, Jerger, & Hall, 1979; Topolska & Hassmann-Poznanska, 2006). Studies are exploring the applicability of MEM reflex testing in the monitoring of pathophysiological changes in the auditory pathways that are associated with blunt head trauma (Nolle, Todt, Seidl, & Ernst, 2004) and industrial noise exposure (Zivic & Zivic, 2003). The MEM reflex is also being studied as a possible addition to the clinical investigations of nonauditory diseases, such as juvenile idiopathic arthritis (Ikiz, Unsal, Kirkim, Erdag, & Guneri, 2007), hydranencephaly (Counter, 2007), amyotrophic lateral sclerosis (Shimizu, Hayashida, Hayashi, Kato, & Tanabe, 1996), myasthenia gravis (Smith & Brezinova, 1991), atypical parkinsonian syndrome (Gironell et al., 2003), and myotonic dystrophy (Osanai, Kinoshita, & Hirose, 2001).

In general, there are five main patterns of interpreting stapedial reflex abnormalities: (a) efferent, (b), afferent (c), central

(brainstem), (d) unilateral (ipsilateral) pattern, and (e) global. The categorization of a specific pattern depends on the presence or absence of the ipsilateral and contralateral reflexes. In an efferent pattern, the stapedial reflex is abnormal in the recorded ear regardless of which ear is stimulated. It may suggest a disruption to the efferent pathway on the same side of the recorded ear similar to that caused by otitis media or a facial nerve abnormality (inactive stapedius). In an afferent pattern, the stapedial reflex is abnormal in the stimulated ear regardless of which ear is being recorded. This pattern signifies a sensorineural hearing loss secondary to an acoustic neuroma affecting the afferent pathway. In a central pathway (brainstem) pattern, all the crossed stapedial reflexes are reduced or absent. This phenomenon is commonly observed in disorders of the brainstem that interfere with the central auditory pathways. Central pathway patterns can also be seen in elderly patients with collapsed ear canals (Schow & Goldbaum, 1980). In the unilateral pattern, all the reflexes are abnormal except for the ipsilateral recording in one ear. This pattern occurs in a middle ear disorder with moderate conductive hearing loss in the recorded ear. It may also suggest a brainstem disorder severe enough to affect the crossed pathways but also the ipsilateral sensory pathway on the side of the recorded ear. The global pattern, in which all reflexes (ipsilateral and contralateral) are abnormal, reflects a severe to profound bilateral hearing loss, bilateral conductive hearing loss, or a central neural disorder affecting the crossed reflex pathways (Bess & Humes, 2008). A better understanding of the MEM reflex circuit diagram would help to better localize a brainstem lesion associated with an abnormal reflex response.

Electrically evoked MEM reflexes have been used in the programming of speech processors in patients with cochlear implants. Recent years have seen a rapid expansion in the technology and general usage of both cochlear and auditory brainstem implantation devices. Monitoring an electrically evoked MEM reflex allows the audiologist to objectively assess the integrity of the peripheral and central auditory brainstem pathways to facilitate the programming of young cochlear implant patients. For instance, measuring the loudness thresholds can be challenging in very young children. Electrically evoked stapedial reflexes are therefore being investigated as an alternative to visual audiometric techniques in the programming of the speech processors in children with cochlear implants (Bordure, O'Donoghue, & Mason, 1996; Caner, Olgun, Gultekin, & Balaban, 2007). Advantages of monitoring an electrically evoked stapedial reflex include providing more comfort to the child while ensuring a reliable replacement to behavioral audiometric techniques in assessing loudness thresholds (Caner et al., 2007; Hodges et al., 1997). Finally, patients with Neurofibromatosis-2 (NF-2) who have a nonviable auditory nerve because of either tumor growth or surgical intervention are ineligible for cochlear implants. Future work on characterizing the auditory brainstem

circuits that comprise the MEM reflexes may lead to objective measures to help guide placement of auditory brainstem electrodes intraoperatively in these NF-2 patients and improve hearing outcomes postoperatively.

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